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(54) Title: PROCESS FOR RINSING FABRICS

(57) Abstract: There is provided a process for rinsing fabrics whereby a benefit agent is delivered to a fabric during the rinse cycle of a washing process, said benefit agent being deposited onto the fabric by means of a reagent having a high binding affinity for the fabric.

WO 01/36577 A1

PROCESS FOR RINSING FABRICSTECHNICAL FIELD

The present invention generally relates to a process for  
5 rinsing fabrics. More in particular, it relates to a process  
for rinsing fabrics whereby a benefit agent is delivered to  
a fabric during the rinse cycle of a washing process.

BACKGROUND AND PRIOR ART

10 A conventional fabric cleaning process as it is carried out  
in an automated washing machine, comprises a main wash cycle  
(which may comprise a number of sub-cycles) in which soiled  
fabrics are contacted with a liquid aqueous cleaning  
composition or wash liquor. Such a composition comprises  
15 surfactants builders and optionally other ingredients such  
as enzymes, bleaching agents, etc. Subsequently, the now  
cleaned fabrics are subjected to a rinse cycle in which the  
cleaning composition is removed by pumping it off and  
spinning the wash load followed by soaking in fresh water.  
20 In the rinse cycle so-called softening agents may be added  
which improve the feel of the washed fabrics. Such softening  
agents conventionally comprise cationic softening compounds  
of the quaternary ammonium type. Other benefit agents  
include perfumes.

25 The compositions which are added during the rinse cycle have  
a relatively short contact time with the fabrics and most of  
the compounds are immediately removed again in the last  
centrifuge step.

30 It is therefore an object of the present invention to  
provide an alternative or improved process for rinsing  
fabrics whereby a benefit agent is delivered to a fabric  
during the rinse cycle of a washing process.

35

Surprisingly, we have now found that these and other objects of the invention may be achieved by the rinsing process of the invention, whereby a benefit agent is delivered to a fabric during the rinse cycle of a washing process, said benefit agent being deposited onto the fabric by means of a reagent having a high binding affinity for the fabric.

WO-A-98/00500 (Unilever) discloses a composition comprising a benefit agent attached to a peptide or protein deposition aid which has a high affinity for fabric. The composition is claimed to effectively deposit the benefit agent onto the fabric during the wash cycle.

According to DE-A-196 21 224 (Henkel), the transfer of textile dyes from one garment to another during a washing or rinsing process may be inhibited by adding antibodies against the textile dye to the wash or rinse liquid.

WO-A-98/07820 (P&G) discloses amongst others rinse treatment compositions containing antibodies directed at cellulase and standard softener actives (such as DEQA).

#### DEFINITION OF THE INVENTION

According to a first aspect of the invention, there is provided a process for rinsing fabrics whereby a benefit agent is delivered to a fabric during the rinse cycle of a washing process, said benefit agent being deposited onto the fabric by means of a reagent having a high binding affinity for the fabric.

According to a second aspect, there is provided a rinse composition for use in the process of the invention, comprising a reagent having a high binding affinity for the fabric and a benefit agent.

DETAILED DESCRIPTION OF THE INVENTION1.1 The benefit agent

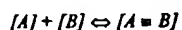
In its first aspect, the invention relates to a process for rinsing fabrics whereby a benefit agent is delivered to a fabric during the rinse cycle of a washing process. The benefit agent can be selected from softening agents, finishing agents/ protective agents, fragrances (perfumes), bleaching agents.

Examples of softening agents are clays, cationic surfactants or silicon compounds. Examples of finishing agents/ protective agents are polymeric lubricants, soil repelling agents, soil release agents, photo-protective agents (sunscreens), anti-static agents, dye-fixing agents, anti-bacterial agents and anti-fungal agents. The fragrances or perfumes may be encapsulated, e.g. in latex microcapsules. Suitable examples of bleaches are photobleaches. Examples of photobleaches are given in EP-A-379 312 (British Petroleum), which discloses a water-insoluble photobleach derived from anionically substituted porphine, and in EP-A-035 470 (Ciba Geigy), which discloses a textile treatment composition comprising a photobleaching component.

1.2 The reagent having a high binding affinity.

In the rinsing process according to the invention, the benefit agent is deposited onto the fabric by means of a reagent having a high binding affinity for the fabric. A specific example of such a reagent is for instance an antibody.

Generally speaking, the degree of binding of a molecule A to another molecule B can be generally expressed by the chemical equilibrium constant  $K_d$  resulting from the following reaction:



The chemical equilibrium constant  $K_d$  is then given by:

$$K_d = \frac{[A][B]}{[A \cdot B]}$$

Whether the binding of a molecule to the fabric is specific or not can be judged from the difference between the binding ( $K_d$  value) of the molecule to one type of fabric, versus the  
5 binding to another type of fabric material. For applications in laundry, said material will be a fabric such as cotton or polyester. However, it will usually be more convenient to measure  $K_d$  values and differences in  $K_d$  values on other materials such as a polystyrene microtitre plate or a  
10 specialised surface in an analytical biosensor. The difference between the two binding constants should be minimally 10, preferably more than 100, and more preferably, more than 1000. Typically, the reagent should bind to the fabric, with a  $K_d$  lower than  $10^{-4}$  M, preferably lower than  
15  $10^{-6}$  M and could be  $10^{-10}$  M or even less. Higher binding affinities ( $K_d$  of less than  $10^{-5}$  M) and/or a larger difference between the one type of fabric and another type of fabric (or background) binding would increase the deposition of the benefit agent. Also, the weight efficiency  
20 of the reagent in the total rinse composition would be increased and smaller amounts of the reagent would be required.

Several classes of reagent or molecules can be envisaged  
25 which deliver the capability of specific binding to fabrics, to which one would like to deliver the benefit agent. In the following we will give a number of examples of such molecules having such capabilities, without pretending to be exhaustive.

30

#### 1.2.1. Antibodies.

Antibodies are specific binding proteins. Their function in nature is to protect against disease by recognising (and binding) foreign bodies, such as viruses or Bacteria, but

not self-cells. Furthermore, methods are well-known in the art to generate antibodies that are specific for almost any protein, organic molecule, or cell surface, that is likely to be encountered. This binding specificity has been exploited in the Biotechnology industry, principally for medical diagnostics. For example, many home-based pregnancy test kits comprise an antibody that specifically binds to the pregnancy marker hormone, human chorionic gonadotropin (hCG), but not to other hormones present in urine.

10

More recently, the use of antibodies in laundry products has been described (Henkel, Procter and Gamble, Unilever). In particular, Unilever has described the use of stain-specific antibodies to target bleaching enzymes exclusively to stains but not to dyes - thus achieving efficient stain removal without damaging surrounding fabric.

15

Antibodies are well known examples of molecules which are capable of binding specifically to compounds against which they were raised. Antibodies can be derived from several sources. From mice, monoclonal antibodies can be obtained which possess very high binding affinities. From such antibodies, Fab, Fv or scFv fragments, can be prepared which have retained their binding properties. Such antibodies or fragments can be produced through recombinant DNA technology by microbial fermentation. Well known production hosts for antibodies and their fragments are yeast, moulds or bacteria.

20

25

A class of antibodies of particular interest is formed by the Heavy Chain antibodies as found in Camelidae, like the camel or the llama. The binding domains of these antibodies consist of a single polypeptide fragment, namely the variable region of the heavy chain polypeptide (HC-V). In contrast, in the classic antibodies (murine, human, etc.), the binding domain consists of two polypeptide chains (the

30

35

variable regions of the heavy chain ( $V_h$ ) and the light chain ( $V_l$ ). Procedures to obtain heavy chain immunoglobulins from Camelidae, or (functionalized) fragments thereof, have been described in WO-A-94/04678 (Casterman and Hamers) and WO-A-  
5 94/25591 (Unilever and Free University of Brussels).

Alternatively, binding domains can be obtained from the  $V_h$  fragments of classical antibodies by a procedure termed "camelization". Hereby the classical  $V_h$  fragment is  
10 transformed, by substitution of a number of amino acids, into a HC-V-like fragment, whereby its binding properties are retained. This procedure has been described by Riechmann et al. in a number of publications (J. Mol. Biol. (1996) 259, 957-969; Protein. Eng. (1996) 9, 531-537,  
15 Bio/Technology (1995) 13, 475-479). Also HC-V fragments can be produced through recombinant DNA technology in a number of microbial hosts (bacterial, yeast, mould), as described in WO-A-94/29457 (Unilever).

20 Methods for producing fusion proteins that comprise an enzyme and an antibody or that comprise an enzyme and an antibody fragment are already known in the art. One approach is described by Neuberger and Rabbits (EP-A-194 276). A method for producing a fusion protein comprising an enzyme  
25 and an antibody fragment that was derived from an antibody originating in Camelidae is described in WO-A-94/25591. A method for producing bispecific antibody fragments is described by Holliger et al. (1993) PNAS 90, 6444-6448.

30 A particularly attractive feature of antibody binding behaviour is their reported ability to bind to a "family" of structurally related molecules. For example, in Gani et al. (J. Steroid Biochem. Molec. Biol. 48, 277-282) an antibody is described that was raised against progesterone but also  
35 binds to the structurally-related steroids, pregnanediolone, pregnanolone and 6-hydroxy-progesterone. Therefore, using

the same approach, antibodies could be isolated that bind to a whole "family" of stain chromophores (such as the polyphenols, porphyrins, or caretenoids as described below). A broad action antibody such as this could be used to treat several different stains when coupled to a bleaching enzyme.

#### 1.2.2. Peptides.

Peptides usually have lower binding affinities to the substances of interest than antibodies. Nevertheless, the binding properties of carefully selected or designed peptides can be sufficient to deliver the desired selectivity in an oxidation process. A peptide which is capable of binding selectively to a fabric to which one would like to deliver a benefit agent, can for instance be obtained from a protein which is known to bind to that specific fabric. An example of such a peptide would be a binding region extracted from an antibody raised against that fabric. A suitable peptide could be analogous to the active center of a protein analogous to a non-catalytic binding domain of a protein, e.g. a receptor.

Alternatively, peptides that bind to such substance can be obtained by the use of peptide combinatorial libraries. Such a library may contain up to  $10^{10}$  peptides, from which the peptide with the desired binding properties can be isolated. (R.A. Houghten, Trends in Genetics, Vol 9, no 6, 235-239). Several embodiments have been described for this procedure (J. Scott et al., Science (1990) **249**, 386-390; Fodor et al., Science (1991) **251**, 767-773; K. Lam et al., Nature (1991) **354**, 82-84; R.A. Houghten et al., Nature (1991) **354**, 84-86).

Suitable peptides can be produced by organic synthesis, using for example the Merrifield procedure (Merrifield (1963) J.Am.Chem.Soc. **85**, 2149-2154). Alternatively, the peptides can be produced by recombinant DNA technology in



microbial hosts (yeast, moulds, bacteria) (K.N. Faber et al. (1996) Appl. Microbiol. Biotechnol. **45**, 72-79).

### 1.2.3. Pepidomimics.

5 In order to improve the stability and/or binding properties of a peptide, the molecule can be modified by the incorporation of non-natural amino acids and/or non-natural chemical linkages between the amino acids. Such molecules are called peptidomimics (H.U. Saragovi et al. (1991) Bio/Technology **10**, 773-778; S. Chen et al. (1992) Proc.Natl.Acad. Sci. USA **89**, 5872-5876). The production of such compounds is restricted to chemical synthesis.

### 1.2.4. Other organic molecules.

15 It can be readily envisaged that other molecular structures, which need not be related to proteins, peptides or derivatives thereof, can be found which bind selectively to fabrics to which one would like to deliver a benefit agent. For example, certain polymeric RNA molecules which have been shown to bind small synthetic dye molecules (A. Ellington et al. (1990) Nature **346**, 818-822). Such binding compounds can be obtained by the combinatorial approach, as described for peptides (L.B. McGown et al. (1995), Analytical Chemistry, 663A-668A).

25

This approach can also be applied for purely organic compounds which are not polymeric. Combinatorial procedures for synthesis and selection for the desired binding properties have been described for such compounds (Weber et al. (1995) Angew.Chem.Int.Ed.Engl. **34**, 2280-2282; G. Lowe (1995), Chemical Society Reviews **24**, 309-317; L.A. Thompson et al. (1996) Chem. Rev. **96**, 550-600). Once suitable binding compounds have been identified, they can be produced on a larger scale by means of organic synthesis.

35

The reagent has a high binding affinity for the fabric. The reagent is a protein or a peptide, it may be that one part of its polypeptide chain is responsible for the binding affinity to the fabric, and part of the reagent comprises an enzyme part capable of providing a benefit. In the first situation, the bleaching enzyme may be a fusion protein comprising two domains, which may be coupled by means of a linker. Alternatively, the reagent having the high binding affinity may be covalently coupled to a benefit agent by means of a bivalent coupling agent such as glutardialdehyde. A full review of chemistries appropriate for coupling two biomolecules is provided in "Bioconjugate techniques" by Greg T. Hermanson, Academic Press Inc (1986). Alternatively, if the reagent having the high binding affinity is a peptide or a protein, it may also be coupled to the enzyme by constructing a fusion protein. In such a construct there would typically be a peptide linker between the binding reagent and the enzyme. An example of a fusion of an enzyme and a binding reagent is described in Ducancel et al. Bio/technology 11, 601-605.

A further embodiment would be for the reagent with a high binding affinity to be a bispecific reagent. Such a reagent could fulfil the requirement of accumulating the benefit agent on the fabric either by supplying said reagent together with the benefit agent as a pre-formed non-covalent complex or by supplying the two separately and allowing them to self-assemble either in the rinse liquor or on the fabric.

In a preferred embodiment, the rinse process of the invention is carried out using micro-particles sensitised with antibody, and configured such that the micro-particles are loaded with the benefit agent and the antibody has a high affinity or specificity for a substance (or "marker molecule") typically found on some regions of fabrics but

not on others. Examples of such marker molecules include bleach-damaged dyes and microbes known to be associated with malodour. The antibody targets the benefit agent to its intended site of action and binds it there. For example,  
5 Microbe-specific antibodies may target fragrance-containing particles to the regions of malodour. Thus, a more efficient use of expensive ingredients is achieved. Alternatively, antibodies specific for bleach-damaged dyes can target dyed particles to faded regions, thus replenishing the colour  
10 lost in the main wash cycle.

Previously, such micro-particles have been sensitised with antibody and used to generate a coloured end-point in medical diagnostic devices, when they are applied manually  
15 onto a test strip. According to the present invention, analogous particles are being specifically bound to some cotton swatches but not others, depending on which marker molecules are present on the swatches. The binding of particles is being driven not by manual application but by  
20 agitating a bulk liquid phase (e.g. a rinse liquor) containing said particles and swatches. The agitation increases the number of collisions between fabric and particles and thus increases specific binding: particles sensitised with specific antibody result in productive  
25 collisions and binding is permanent; particles sensitised with non-specific antibody result in non-productive collisions and do not bind permanently. Such an agitation could be readily achieved during the rinse cycle in an automatic washing machine.

30

Another advantage of the present invention is that it is possible to target some benefit molecules to particular regions of fabric during the rinse. For example, dyes can be targeted to colour-bleached regions to replenish dye lost in  
35 the main wash or fragrance can be targeted to regions where it is most needed (i.e. those regions where microbes

associated with malodour are present - such as the "underarm" regions). However, methods for targeting small molecules (such as a dye or a fragrance) to particular regions of fabric have not previously been described. The inventors have approached this problem by loading small molecules (such as a dye) onto a micro-particle and then sensitising the particles with an antibody. The advantage of this is that a single antibody binding event can deposit many dye molecules onto the target-region of fabric. Whereas antibody-sensitised particles have been described previously (as component parts of medical diagnostic devices) they are used in a fundamentally different way: in the medical device, the particles are manually applied to the target surface (typically nitro-cellulose paper) and then eluted with a solution. If specific antibody is present, the particles remain stuck on. Otherwise they do not. In contrast, antibody-sensitised particles have not previously been used to target a small chemical compound (such as a dye or a fragrance) from a bulk liquid phase to a particular target site on a surface. Furthermore, if an analogous interaction between the particles and the target surface (i.e. if the swatches are manually placed in the bulk liquid and left static) little or no binding is observed. However, the inventors have been able to achieve surprisingly specific binding to cotton swatches by agitating a bulk liquid phase (of rinse liquor or tap water) containing said particles and swatches.

### 1.3 The fabrics

For laundry detergent applications, several classes of natural or man-made fabrics can be envisaged, in particular cotton. Such macromolecular compounds have the advantage that they can have a more immunogenic nature, i.e. that it is easier to raise antibodies against them. Furthermore, they are more accessible at the surface of the fabric than

for instance coloured substances in stains, which generally have a low molecular weight.

An important embodiment of the invention is to use a binding reagent (as described above) that binds to several different types of fabrics. This would have the advantage of enabling a single benefit agent to be deposited to several different types of fabric.

## 10 2. The Rinse Composition.

The rinse compositions of the invention can be used in a detergent composition which is specifically suited for rinsing purposes, and this constitutes a second aspect of the invention. When formulating a rinse product, it is important to ensure that the other ingredients of the product are compatible with antibody activity. WO-A-98/07820 (P&G) discloses *inter alia* rinse treatment compositions containing antibodies directed at cellulase and standard softener actives such as DEQA. The rinse product according to the present invention preferably contains no softener or low levels of softener active (e.g. HEQ). If HEQ is present, the rinse product contains Sodium tripolyphosphate (STP) to stabilise antibody activity.

25 However, the present inventors achieved much superior binding and specificity in rinse liquors (or tap water) by omitting typical softener compositions. They also achieved improved binding in the presence of softener compositions by adding salts, especially multivalent salts such as STP. It is also very surprising that the inventors have found antibodies to be active in rinse liquors (or tap water). Previously published descriptions of specific antibody binding are typically in physiological strength buffer (0.15M NaCl) often supplemented with 0.15% surfactant. In many ways this mimics the environment in which the antibodies bind in nature, namely in serum which is

approximately 0.15M NaCl, pH 7, and where serum albumin may be thought to act in an analogous way to a surfactant in that it reduces the opportunity for non-specific binding reactions.

5

To that extent, the rinse composition comprises one or more benefit agents and optionally other conventional detergent ingredients. The invention in its second aspect provides a rinse composition which comprises from 0.1 - 50 % by weight, based on the total composition, of one or more surfactants. This surfactant system may in turn comprise 0 - 95 % by weight of one or more anionic surfactants and 5 - 100 % by weight of one or more nonionic surfactants. The surfactant system may additionally contain amphoteric or zwitterionic detergent compounds, but this is not normally desired owing to their relatively high cost. It was found to be advantageous to also include cationic surfactants into the composition. Examples of suitable cationic surfactants are given in WO-A-97/03160 and WO-A-98/17767 (Procter&Gamble).

20

In general, the nonionic and anionic surfactants of the surfactant system may be chosen from the surfactants described "Surface Active Agents" Vol. 1, by Schwartz & Perry, Interscience 1949, Vol. 2 by Schwartz, Perry & Berch, Interscience 1958, in the current edition of "McCutcheon's Emulsifiers and Detergents" published by Manufacturing Confectioners Company or in "Tenside-Taschenbuch", H. Stache, 2nd Edn., Carl Hauser Verlag, 1981.

Suitable nonionic detergent compounds which may be used include, in particular, the reaction products of compounds having a hydrophobic group and a reactive hydrogen atom, for example, aliphatic alcohols, acids, amides or alkyl phenols with alkylene oxides, especially ethylene oxide either alone or with propylene oxide. Specific nonionic detergent compounds are C<sub>6</sub>-C<sub>22</sub> alkyl phenol-ethylene oxide condensates,

generally 5 to 25 EO, i.e. 5 to 25 units of ethylene oxide per molecule, and the condensation products of aliphatic C<sub>8</sub>-C<sub>18</sub> primary or secondary linear or branched alcohols with ethylene oxide, generally 5 to 40 EO.

5

Suitable anionic detergent compounds which may be used are usually water-soluble alkali metal salts of organic sulphates and sulphonates having alkyl radicals containing from about 8 to about 22 carbon atoms, the term alkyl being used to include the alkyl portion of higher acyl radicals. Examples of suitable synthetic anionic detergent compounds are sodium and potassium alkyl sulphates, especially those obtained by sulphating higher C<sub>8</sub>-C<sub>18</sub> alcohols, produced for example from tallow or coconut oil, sodium and potassium alkyl C<sub>9</sub>-C<sub>20</sub> benzene sulphonates, particularly sodium linear secondary alkyl C<sub>10</sub>-C<sub>15</sub> benzene sulphonates; and sodium alkyl glyceryl ether sulphates, especially those ethers of the higher alcohols derived from tallow or coconut oil and synthetic alcohols derived from petroleum. The preferred anionic detergent compounds are sodium C<sub>11</sub>-C<sub>15</sub> alkyl benzene sulphonates and sodium C<sub>12</sub>-C<sub>18</sub> alkyl sulphates. Also applicable are surfactants such as those described in EP-A-328 177 (Unilever), which show resistance to salting-out, the alkyl polyglycoside surfactants described in EP-A-070 074, and alkyl monoglycosides.

Preferred surfactant systems are mixtures of anionic with nonionic detergent active materials, in particular the groups and examples of anionic and nonionic surfactants pointed out in EP-A-346 995 (Unilever). Especially preferred is surfactant system which is a mixture of an alkali metal salt of a C<sub>16</sub>-C<sub>18</sub> primary alcohol sulphate together with a C<sub>12</sub>-C<sub>15</sub> primary alcohol 3-7 EO ethoxylate.

The nonionic detergent is preferably present in amounts greater than 10%, e.g. 25-90% by weight of the surfactant

system. Anionic surfactants can be present for example in amounts in the range from about 5% to about 40% by weight of the surfactant system.

- 5 The rinsing detergent composition may take any suitable physical form, such as a powder, a tablet, an aqueous or non aqueous liquid, a paste or a gel. The complex of benefit agent and reagent having a high affinity according to the invention will generally be used as a dilution in water of  
10 about 0.05 to 2%.

The rinse composition in accordance with the invention comprising the complex of the reagent having a high affinity for the fabric and the benefit aid can have any suitable  
15 form, i.e. the form of a granular composition, a liquid or a slurry of the enzyme, or with carrier material (e.g. as in EP-A-258 068 and the Savinase (TM) and Lipolase (TM) products of Novo Nordisk). A good way of adding the complex to a liquid rinse product is in the form of a slurry  
20 containing from 0.005 to 50 % by weight of the complex in an ethoxylated alcohol nonionic surfactant.

The rinse compositions of the invention comprise about 0.001 to 10 mg, preferably from 0.01 to 10 mg of antibody per  
25 liter of the rinse liquor in use. A concentrated rinse composition before use will comprise about 1 to 1000 mg/l, preferably from 10 mg to 100 mg per liter of the rinse product.

30 In the figures is:

**Figure 1** a Graph showing the effects of wash and rinse liquors from Persil non-biological powder main wash on antibody binding activity (a specific anti-hCG monoclonal was used) compared to standard curves in PBST and tap water  
35 bench marks). Graph shows binding signal (optical density at 405nm) versus antibody concentration.



Figure 2: Graph showing the effects of wash and rinse liquors from Zeus liquid main wash on antibody binding activity (a specific anti-hCG monoclonal was used) compared to standard curves in PBST and tap water bench marks. Graph 5 shows binding signal (optical density at 405nm) versus antibody concentration.

The invention will now be further illustrated in the following, non-limiting Examples.

10

#### Example 1

##### **Antibody binding in Tap water to antigen.**

Method: Antibody activity was determined using an immuno-assay or 'ELISA'. The assay comprised a specially designed 15 nylon peg sensitised with antigen. A number of wash and rinse liquors were used to determine the effects on antibody activity. These liquors were obtained from wash cycles using standard Persil non-biological powder and Zeus (concentrated zeolite built) non-biological HDL formulation.

20

All reagent solutions were exposed to the peg surface in microtitre plates. The nylon pegs were first washed with ethanol, prior to activation of the peg surface with gluteraldehyde. The gluteraldehyde was then washed away with 25 distilled water before sensitising the surface of the pegs with hCG, and blocking with bovine serum albumin. The blocked pegs were 'crunched' onto plastic bars, twelve pegs per bar, and sucrose coated to preserve them before drying and sealing in pouches for storage. Firstly, the pegs had to 30 be rehydrated by immersing in phosphate buffer saline (PBS) [0.15M sodium chloride, 0.0075M di-sodium hydrogen orthophosphate and 0.0025M sodium di-hydrogen orthophosphate, pH7.2] or distilled water only if using tap water or wash/rinse liquor as antibody diluent, for 15 minutes. Mouse 35 anti-hCG was assayed as a dilution curve, with concentrations starting at 1µg/ml, in phosphate buffered

saline + 0.015% v/v tween 20 (PBST) pH7.2. Mouse anti-E3G was assayed as a negative control to allow assessment of non-specific binding and was also run in a dilution curve, with equal concentrations to the mouse anti-hCG. The pegs  
5 were exposed to the various concentrations of anti-hCG and anti-E3G for 60 minutes, at room temperature, before washing in PBST. This was followed by exposure to Goat anti-mouse alkaline phosphatase conjugate, diluted in PBST, for 30 minutes at room temperature. After a final wash in PBST, the  
10 pegs were analysed for the amount of alkaline phosphatase bound by incubating in a colour generating substrate system (p-nitro-phenyl phosphate) where the more antibody bound the more colour is generated. The resulting colour was read in an ELISA plate reader at 405nm. Figures 1 and 2 demonstrate  
15 the ability of the antibodies to bind to its antigen in tap water and rinse liquors.

### Example 2

Binding antibody loaded particles to fabric sensitised with  
20 antigen.

Method: Two centimetre squares of cotton fabric were treated with cellulase/hCG conjugate (to sensitise fabric with antigen) and were then exposed to specific and non-specific antibody sensitised latex particles, diluted to 0.1% solids  
25 in PBS + 0.2% non-ionic surfactant Co-Co 6.5EO and tap water. Exposure was static, for 60 minutes, at room temperature. The exposed cotton squares were then washed thoroughly with large volumes of tap water and vigorous shaking.

30 Results: The results presented in the table below indicate the ability to target particles to an antigen on fabric via a specific antibody binding event in tap water. The greater the DE the greater the particle deposition.

**Table 1.** Binding of targeted particles to cotton in tap water (non-shaking/static exposure)

Cotton Surface Treatment	Particle Sensitisation	Cotton/ Particle interaction	$\Delta E$ values	$\Delta$ Reflectance ( $\Delta R$ ) at 650nm
Cellulase/hCG	Specific antibody (anti-hCG)	Targeted	9.0	20.8
No conjugate	Specific antibody (anti-hCG)	Untargeted	1.2	2.9
Cellulase/hCG	Non-specific antibody (anti-E3G)	Untargeted	1.9	4.7
No conjugate	Non-specific antibody (anti-E3G)	Untargeted	1.5	0.8

- 5 The examples illustrated above relate to monoclonal antibody molecules. The following examples demonstrate similar effects using antibody fragments.

### Example 3

#### 10 **Functional bihead antibody molecule in tap water.**

This example describes the confirmation that engineered antibody molecules (with the potential of large-scale exploitation) can function effectively in a non-buffered tap water environment.

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Method: Bi-head molecule (denoted 1349) will bind red wine stains (anti-polyphenolic) and the enzyme Glucose oxidase (Gox). Bi-Head 1349 was diluted at 10 $\mu$ g/per ml in tap water

or Phosphate buffered saline + tween 20, PBST [0.01M  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ , 0.15M NaCl and 0.15% Tween 20] each containing 5µg/ml Gox. Additionally a control of Gox only at 5µg/ml was diluted in tap water or PBST to be used as a control. The prepared solutions were dispensed in 1ml volumes to two stained swatches and two unstained swatches. Tap water and PBST only were added to two unstained swatches as a further control. Samples were left at 21°C(± 1) for 30 minutes without shaking. The samples were placed in bottle containing either tap water or PBST (as appropriate) and agitated on a rotatorque (Cole Parmer instrument Co. Chicargo Model 7637-10) for 2 minutes. This rinsing process was repeated twice more. The swatches were then placed in a clean 24 well tissue culture plate and a substrate was added comprising 0.1M Na Phosphate buffer pH 6.5, 10mM Glucose, 1µg/ml Horse Radish Peroxidase [Sigma] and 10µg/ml Tetramethyl Benzidine [Sigma]. This would produce a coloured endpoint that was proportional to the amount of Gox present on the cloth. To stop the reaction 300µl of 1M HCL was added to each well, samples of 100µl were then transferred to microtitre plate wells (Sterilin) and the absorbance at 450nm was measured with a plate reader (Dynatech MR5000). The results are:

	1349/Gox	Gox
Tap water/Wine	2.065	1.26
PBST/Wine	1.511	0.002
Tap water /Unstained	0.258	0.043
PBST/ Unstained	0.002	0.001

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The data show that the binding signal in tap water on red wine stain by 1349+Gox is approximately twice that of Gox when it is untargeted. For a comparison the signal in PBST for untargeted Gox is less than 0.01 whereas that for

30

- 1 1349+Gox (targeted) is 150 times greater. There is an  
increase in the non-specific binding of Gox to stained cloth  
in tap water. However, on unstained cloth the background  
signal for 1349+Gox is small and the signal to noise ratio  
5 is high at 8:1, this is acceptable and demonstrates  
significant specific binding by the bi-head molecule to the  
red wine stain. The controls for tap water and PBST only  
resulted in no signal.

CLAIMS

1. Process for rinsing fabrics whereby a benefit agent is delivered to a fabric during the rinse cycle of a washing  
5 process, said benefit agent being deposited onto the fabric by means of a reagent having a high binding affinity for the fabric.
2. Process according to claim 1, wherein the benefit agent  
10 is selected from the group consisting of softening agents, finishing agents/ protective agents, fragrances or perfumes and bleaching agents.
3. Process according to any one of the preceding claims,  
15 wherein the reagent having a high binding affinity for the fabric is a protein or a peptide.
4. Process according to any one of the preceding claims,  
20 wherein the reagent having a high binding affinity is an antibody, an antibody fragment, or a derivative thereof.
5. Process according to any one of the preceding claims,  
wherein the reagent having a high binding affinity has a chemical equilibrium constant  $K_d$  for the fabric of less than  
25  $10^{-4}$  M, preferably less than  $10^{-6}$  M.
6. Process according to claim 5, wherein the chemical equilibrium constant  $K_d$  is less than  $10^{-7}$  M.
- 30 7. Process according to any one of the preceding claims, wherein the fabric is cotton, polyester, or polyester/cotton.
8. Process according to any one of the preceding claims,  
35 wherein the reagent having a high binding affinity is directed at a specific part of the fabric.

9. Process according to any one of the preceding claims,  
using micro-particles sensitised with antibody, and  
configured such that the micro-particles are loaded with the  
5 benefit agent.

10. Process according to any one of the preceding claims,  
whereby the reagent having a high binding affinity for the  
fabric is a multi-specific antibody or antibody or an  
10 analogous structure arranged so that at least one  
specificity is directed to the fabric and the others are  
directed to one or more benefit agents.

11. Process according to claim 9, wherein the reagent has  
15 one specificity directed to the fabric and one to the  
benefit agent.

12. Rinse composition for use in the process according to  
claims 1-11 comprising a reagent having a high binding  
20 affinity for the fabric and a benefit agent.

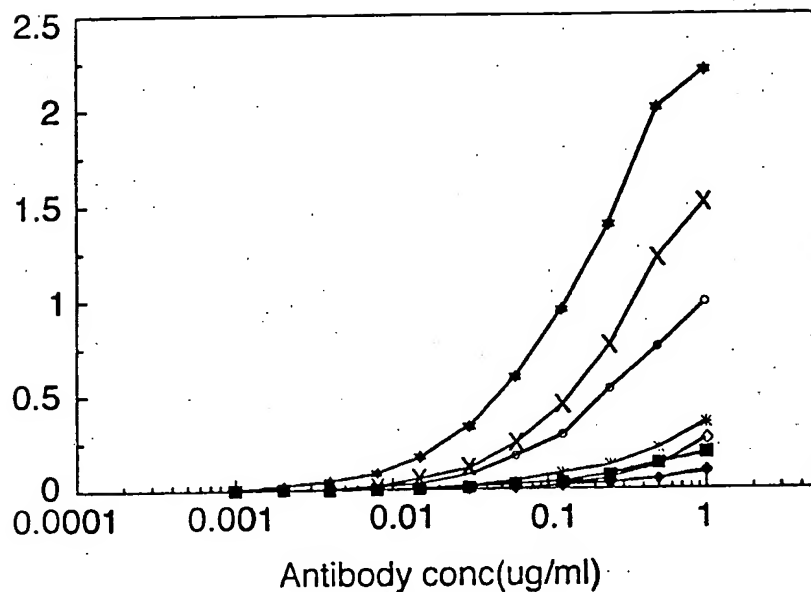
13. Rinse composition according to claim 12, using micro-  
particles sensitised with antibody, and configured such that  
the micro-particles are loaded with the benefit agent.

25

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Fig.1.

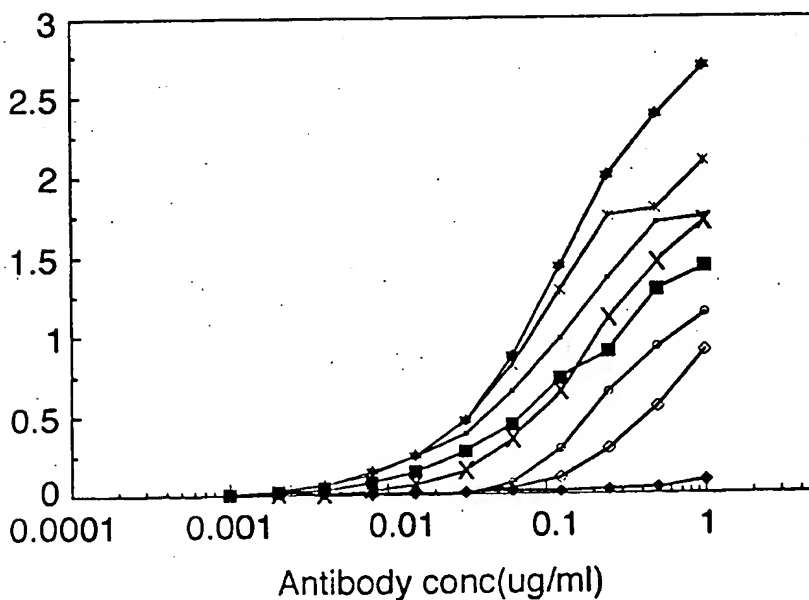
Binding 'Signal'



PBST  
 Tap Water  
 Tap Water  
 +0.6% Comfort  
 Wash Liquor  
 (pH10.3)  
 Rinse 1  
 Rinse 2  
 Rinse 3  
 (pH7.7)  
 Rinse 4  
 (incl. Comfort)

Fig.2.

Binding 'Signal'



PBST  
 Tap Water  
 Tap Water  
 +0.6% Comfort  
 Wash Liquor  
 (pH 7.3)  
 Rinse 1  
 Rinse 2  
 Rinse 3  
 (pH6.7)  
 Rinse 4  
 (incl. Comfort)



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/10912

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 7 C11D3/384 C11D3/00 C11D3/50		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 7 C11D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) WPI Data		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 56885 A (UNILEVER PLC ;UNILEVER NV (NL)) 17 December 1998 (1998-12-17) examples 4,16,17; tables 1,2 ---	1-13
X	WO 98 00500 A (UNILEVER PLC ;UNILEVER NV (NL)) 8 January 1998 (1998-01-08) cited in the application page 5, line 4 - line 12; claims 16-19; example 2 ---	1-3,5,7, 10-12
X	DATABASE WPI Section Ch, Week 199920 Derwent Publications Ltd., London, GB; Class A23, AN 1999-233454 XP002161477 & JP 11 061639 A (DAINIPPON JOCHUGIKU KK), 5 March 1999 (1999-03-05) abstract ---	1-3,12
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <span style="margin-left: 100px;"><input checked="" type="checkbox"/> Patent family members are listed in annex.</span>		
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>* Special categories of cited documents:</p> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>*Z* document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">27 February 2001</div>		Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">08/03/2001</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer <div style="text-align: center; font-weight: bold;">Loiselet-Taisne, S</div>

# INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/EP 00/10912

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 98 07821 A (WEVERS JEAN ;HAUWERMEIREN TIM MARIA JORIS V (BE); HERBOTS IVAN MAU) 26 February 1998 (1998-02-26) examples 14,15	1-7,12

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